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The Production of Antibodies by Isolated Spleen Cells Following Contact with an Antigen in vitro

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The latest communications dealing with the possibility of antibody formation in tissue cultures to which an antigen has been added in vitro show that antibodies are not formed under these conditions (Parker 1937, Salle and McOmie 1937, Selmar 1944, Fastier 1948). Positive antibody formation in tissue cultures can be demonstrated only if the tissue used for culturing is taken from an animal which has been immunised in vivo (Meyer and Loewenthal 1927, Parker 1937, Fagraeus 1948a, Ranney and London 1951, Thorbecke and Keuning 1953, Tanaka 1953, Stavitsky 1955, Askonas and Humphrey 1955). These results give evidence, as concluded by Parker (1937), that the first phase of the reaction to an antigen takes place only under the conditions of the organism.

It was assumed by the author that the non-specific mobilisation reaction which occurs in the organism following administration of an antigen, directly participated in antibody formation. This reaction is displayed both in biochemical and physiological changes (changes in the blood sugar level, temperature, the number of leucocytes, etc.) and also in changes of a morphological character in the mesenchymal tissue (Fagraeus 1948b, Marshall and White 1950, Makinodan et al. 1954).

The present communication attempts to establish to what degree antibody formation is dependent on the non-specific metabolic and morphological reaction of the organism following administration of the antigen. The metabolic and morphological changes were produced by a different antigen (guinea-pig serum); after a given time (24 to 96 hours), the spleen was removed and the spleen cells isolated and mixed with the antigen (*Salmonella paratyphi B*) in vitro. The mixture of spleen cells and antigen was then administered intraperitoneally to young rabbits. These had already proved to be the best animals for the transfer of immunised tissues in previous experiments, as very young animals do not respond to an antigen by antibody formation (Šterzl 1955a). The same procedure was used with the controls, in which the spleen cells of a normal, non-immunised animal were mixed with the antigen.

Methods

A non-specific antigenic stimulus was produced by injecting rabbits (2—3 kg.) intravenously with 1 ml. guinea-pig serum 24—96 hours prior to killing, as described in the individual experiments.

The spleen of both immunised and normal rabbits was removed and prepared in a cooled room at 2° C. The cells were expressed from the spleen capsule into a chilled phosphate-physiological saline solution with 0.2% gelatine. The individual cells were freed by repeated sucking into a pipette. For further washing, which was carried out three times in the same solution, only a homogenous suspension of cells was used. The final dilution was made by adding 1 ml. of fluid (suspension of antigen, in the controls physiological saline) to 0.05 g. splenic tissue. In every experiment the number of cells was determined

in a Bürker chamber; a suspension of cells prepared in this way contained on an average $30-40 \times 10^6$ lymphoid cells/1 ml.

The antigen used was a heat-inactivated suspension of *S. paratyphi B*. The quantitative inter-relationship of spleen cells and antigen was one of the decisive factors for the experiment and is given in the results. The mixture of spleen cells and antigen was incubated at 37 °C and then injected intraperitoneally in doses of 1 ml. in 5-day-old rabbits. The incubation time varied in the different experiments and precise details are given in the tables; in most cases the time of mixing was 10 minutes.
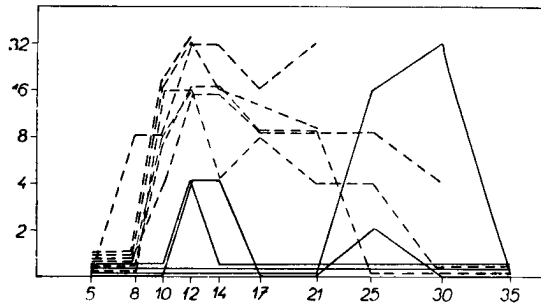
The times at which blood was collected from the young rabbits by cardiac puncture are also given in the tables. After collection, the sera were stored at $-15°$ C and agglutination was carried out in every group at the same time. In cases where it was necessary to remove lipoid substances, the sera were shaken out with chloroform (Šterzl 1955). The agglutination specimens were stored in a refrigerator and the results read off after 4, 6 and 8 days.

The cells were irradiated in a dish placed in ice, in a layer not exceeding 1 mm. Irradiation was carried out using a Mikrometa apparatus (AEG — 50-X-ray tube) under the following conditions: focal distance 10 cm., Al 0.1, KV 50, mA 6. With these constants the concentration of irradiation is 430 r/10 seconds.

Fig 1. Transfer of spleen cells of normal rabbit $(40 \times 10^6$ cells/1 ml.) to two groups of young rabbits; mixed for 10 minutes: a) dashed — with antigen *S paratyphi B* in concentration of 100×16^6 microorganisms/1 ml., b) full — with antigen 500×10^6 micro-organisms/1 ml. x: age of rabbits in days; days on which blood collected denoted. y: titre of agglutinating antibodies.
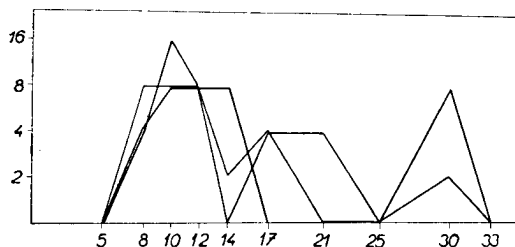

Fig. 2. Isolated spleen cells $(32 \times 10^6/1$ ml.) of rabbit immunised intravenously 24 hours previously with 1 ml. guinea-pig serum. The cells were mixed in vitro with the antigen (10^8 micro-organisms/ 1ml.) for 10 minutes. x and y: as in tab. 1.

Results

In the first experiments (Šterzl 1955), in which the cells of a normal animal were mixed with an antigen and transferred intraperitoneally to young rabbits, no antibody formation was found. In these experiments the amount of antigen used was 10^9 bacterial cells in 1 ml. On reducing the amount of antigen added to the cells in vitro, conclusive evidence of antibody formatiom was found after a transfer to young rabbits. The optimal relationship between antigen and cells was therefore investigated. The results obtained hitherto show that the most satisfactory proportion is $1-2$ micro-organisms to one spleen cell. A higher concentration of the antigen inhibits antibody formation (fig. 1).

Antibody formation was found after mixing the antigen *(S. paratyphi B.)* with spleen cells isolated from animals following non-specific stimulation with guinea-pig serum. The experiments were carried out in 60 young rabbits from 10 litters. In all groups where there had been antigenic stimulation by the administration of a foreign serum $24-96$ hours before removal of the spleen, followed by mixture of the spleen cells with the antigen *(S. paratyphi B)* in vitro, antibodies which agglutinated the specific antigen were found within a few days following intraperitoneal transfer to young rabbits (fig. 2 and 3). No substantial increase in antibody formation was found even when the cells were isolated from the spleen of an animal which had

2

been immunised over a period of 14 days with five doses of guinea-pig serum (fig. 4). This finding is of interest in view of the fact that during the first days after non-specific immunisation an increasingly marked morphological reaction took place in the spleen tissue in all experiments (an increase in the number of reticular cells, plasmoblasts and plasmocytes and a transitory decrease in the lymphocytes — Holub 1957). Antibody formation was also demonstrated in experiments in which spleen cells were isolated from an organism immunised in vivo with the specific antigen *(S. paratyphi B)* and mixed with the antigen in vitro (fig. 5).
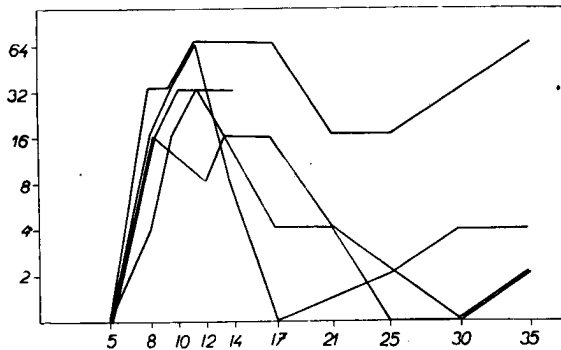

Fig. 3. Isolated spleen cells (41 × 10⁶/1 ml.) of rabbit immunised intravenously 72 hours previously with 1 ml. guinea-pig serum. Incubated in vitro with antigen (10⁸ micro-organisms/1 ml.) for 10 minutes and transferred intraperitoneally to group of young rabbits. x and y: as in tab. 1.
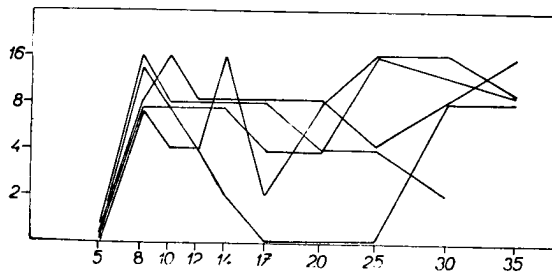

Fig. 4. Spleen cells were isolated from the spleen of rabbit No. 43 (35 × 10⁶/1 ml.), which had been immunised over a period of 14 days with five intravenous doses of 1 ml. guinea-pig serum. These were mixed for 10 minutes in vitro with the antigen 10⁸ micro-organisms/1 ml.) and after incubation were transferred intraperitoneally to a group of young rabbits.
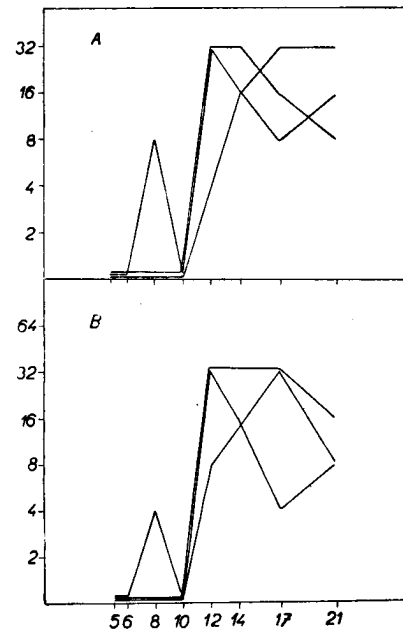

Fig 5. Isolated spleen cells (45 × 10⁶/1 ml.) from two rabbits immunised intravenously 72 hours previously with 1 ml. antigen *S. paratyphi B* (10⁸ micro-organisms). Half the cells transferred to young rabbits A) only washed in physiological saline, B) incubated, after washing, with antigen for 10 minutes (10⁸ micro-organisms/1 ml.). x and y: as in tab. 1.

In further experiments carried out in eight groups of young rabbits, spleen cells from normal animals which were mixed with the optimal amount of antigen were transferred to the young animals. In all these experiments also there was clear evidence of the formation of agglutinating antibodies (fig. 6).

Since spleen cells prepared in the same way and mixed with the optimal amount of antigen did not form antibodies when cultured in tissue cultures (Rychlíková and Šterzl 1957), it was necessary to consider whether the young rabbits did not participate actively in the formation of antibodies. It was demonstrated, however
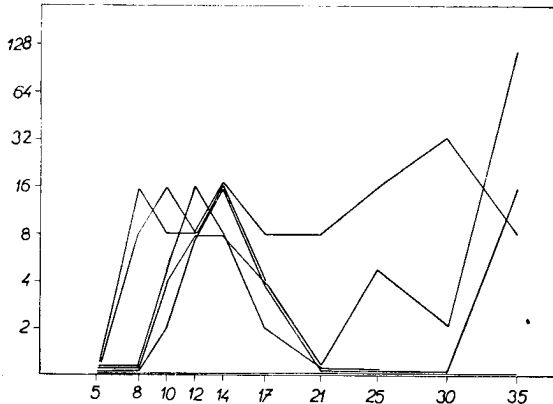
3

Fig. 6. Isolated cells from normal rabbit (44 × 10⁶/1 ml.), mixed with antigen (10⁸/1 ml.) for 10 minutes. *x* and *y*: as in tab. 1.
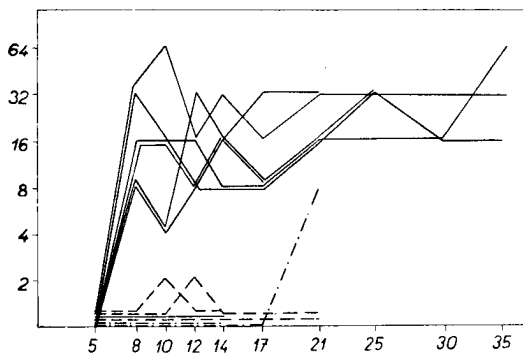

Fig. 7. Isolated spleen cells of normal rabbit (39 × × 10⁶/1 ml.) and irradiated with 860 r (full), 1,000 r (dashed) and 1,200 r (dash and dot), then mixed with antigen (10⁸ micro-organisms/1 ml.) for 10 minutes and injected intraperitoneally in young rabbits. *x* and *y*: as in tab. 1.
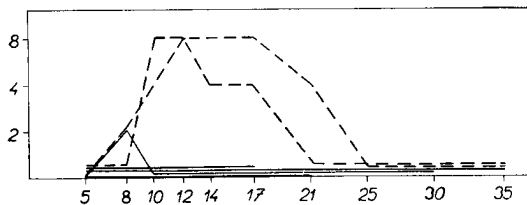

Fig. 8. Isolated spleen cells (43 × 10⁶/1 ml.). Half the cells not irradiated (dashed line), half irradiated with 1,200 r (full line). After 4 hours incubation at 37°C mixed with antigen (10⁸ micro-organisms) and injected intraperitoneally in young rabbits.

in eight groups (55 animals) that if the isolated cells are irradiated, antibodies were formed only in those animals to which cells not damaged by irradiation had been administered. The cells which go on to form antibodies are either those which have received only small doses of irradiation (fig. 7) or non-irradiated cells (fig. 8). Doses of 1,000 and 1,200 r completely inhibit antibody formation.

The possibility of the active participation of the young animals in antibody formation is likewise not supported by the following finding: After transfer of the cells, an initial phase of rapid development of antibodies occurs, which can be inhibited by irradiating the cells. The second phase of the antibody reaction is the active response of the young animals to the antigen which is transferred together with the cells, and coincides chronologically with the development of antibodies which takes place when only the antigen is administered to young animals (20th—30th day of life). In the transfer of cells together with the antigen, therefore, the question is not one of induction of the active response of the young animals to the antigen, as in that case active antibody formation would have to commence shortly after transfer of the cells. Nor could it be demonstrated that the administration of a foreign antigen in any way accelerated the response of the young animals to the antigen. This was demonstrated by an experiment in which a different antigen (guinea-pig serum) was first injected and the antigen *S. paratyphi B.* was injected seven days later. The formation of antibodies was in no way speeded up following this measure, as compared with the controls (fig. 9). The author regards the organism of the young rabbits to which the spleen cells are transferred, together with the antigen, as passive, as a suitable culture medium encouraging the development of the cells and their complex biochemical processes which participate in the formation of the antibodies.

4

It was also wished to determine whether active processes take place in spleen cells in the course of short-term incubation of the antigen with the cells in vitro. It is possible that the antigen and spleen cells are simply transferred into a suitable environment and that the actual reaction of antibody formation takes place only within the young animal. There is evidence for this possibility in the fact that if spleen cells from a normal adult rabbit were injected in young rabbits and the antigen was not injected until 24, 48 and 72 hours later (intraperitoneally), an antibody reaction also occurred (fig. 10). This would indicate that the transferred spleen cells survive in the young rabbit and that contact of the antigen with them is also possible in vivo. Antibody formation was also demonstrated in cases in which the spleen cells
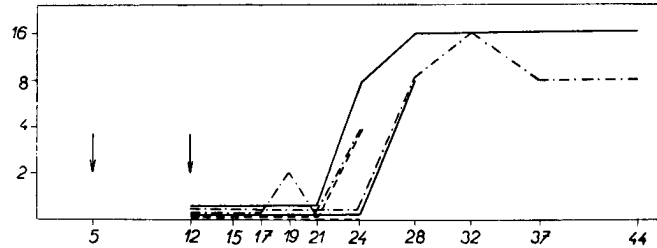

Fig. 9. From a group of six young rabbits, four injected on the fifth day of life with guinea-pig serum. Dash-dot: 2 ml. guinea-pig serum intraperitoneally. Dashed: 3 ml. guinea-pig serum. Full: controls without serum. Antigen (10^8 micro-organisms/1 ml.) administered by intracardiac injection on 12th day of life. x and y: as in tab. 1.

were first injected intraperitoneally and the antigen was then injected into the blood stream (intracardially — fig. 11). This experiment also indicates that cells transferred intraperitoneally do not remain only locally, but that they find their way into the internal organs of the animal, as demonstrated by Holub (1957).

An attempt was made to demonstrate the significance of the time for which the cells are in contact with the antigen in vitro by washing out the antigen after incu-
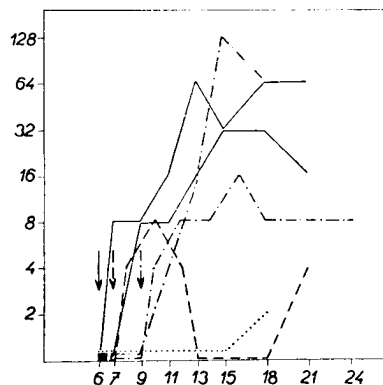

Fig. 10. Isolated spleen cells (28 × 10^6/1 ml.) injected intraperitoneally in young rabbits. Full: cells mixed immediately with 1 ml. antigen. Dashed: 1 ml. antigen injected intraperitoneally 24 hours after injection of spleen cells. Dash-dot: 1 ml antigen injected intraperitoneally 72 hours after injections of spleen cells. Dotted: spleen cells killed by heating to 56° C for 30 minutes and injected in young rabbits after being mixed with antigen. Concentration of antigen: 10^8 micro-organisms/ 1 ml. x and y: as in tab. 1

bation together with the cells in vitro. In these experiments the cells were transferred to four groups of young animals (21 in all). It was seen that washing out of the superfluous antigen did not destroy the ability of the transferred cells to form antibodies (fig. 12). The amount of antigen remaining in the cells was determined, following destruction of the cells by freezing and thawing, by immunising adult animals. In adult animals the amount of antigen added to the cells (10^8 micro-organisms) gives an antibody titre of 1 : 512—1,024. Following immunisation with destroyed cells, the antibody titre in rabbits averaged 1 : 32—64. Cells were trans-
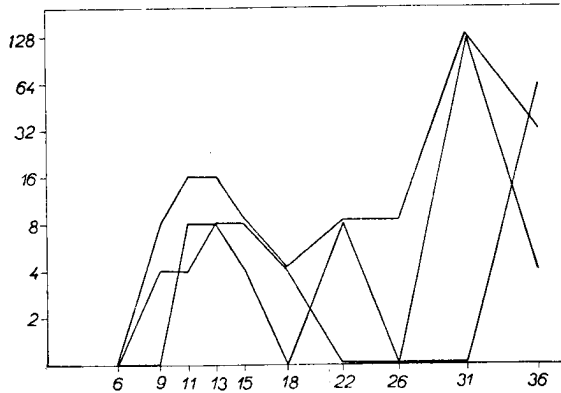
5

Fig. 11. Isolated spleen cells of rabbit immunised 72 hours previously with guinea-pig serum. After washing, the cells were suspended in physiological saline ($35 \times \times 10^6$ micro-organisms/1 ml.) and 1 ml. injected intra-peritoneally. The antigen (10^8 micro-organisms) was then injected into the blood stream by the intracardiac route. x and y: as in tab. 1.
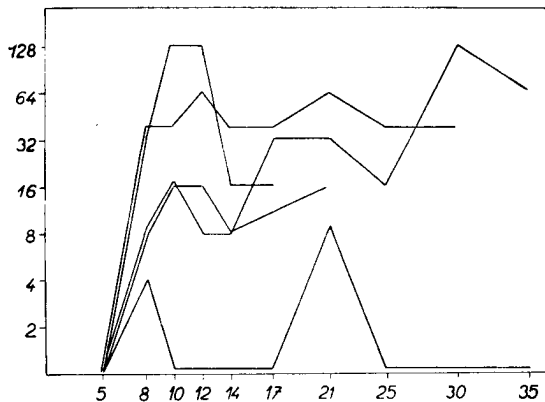

Fig. 12. Spleen cells isolated from normal rabbit ($39 \times \times 10^6$ micro-organisms/1 ml.) incubated together with antigen (10^8 micro-organisms/1 ml.) for two hours at $37°$ C. After incubation superfluous antigen washed out with gelat. physiological saline and cells injected intra-peritoneally in doses of 1 ml. in group of young rabbits. Part of the cells destroyed by freezing and thawing to determine the amount of antigen in the cells. x and y: as in tab. 1.

ferred to two groups of young rabbits (13 in all); half of these cells had been incubated, together with the antigen, in a thermostat and half in a refrigerator. Following incubation the cells were washed and it was found that the cells incubated together with the antigen in the refrigerator did not form antibodies. Although it is not possible to conclude from these experiments that the initial biochemical processes of antibody formation take place already in vitro, the results permit the conclusion that in vitro the cells bind the effective amount of antigen, which is only part of the total amount added.

Discussion

Antibody formation was obtained on isolated spleen cells by mixing them in the optimal proportion with the antigen in vitro and transferring them after incubation intraperitoneally to young rabbits. At the time of our first communication on the successful formation of antibodies by isolated spleen cells (Šterzl and Hrubešová 1955b), an extensive work by Harris et al. was published (1955), preceded by a preliminary communication (1954). Harris et al. succeeded in obtaining antibody formation on isolated cells of the lymphatic glands, mixed with an antigen and transferred to animals which had undergone X-ray irradiation. Although a different form of experiment and a different antigen were used, the results are basically the same. Roberts and Dixon on the other hand (1955), were unable to demonstrate antibodies by mixing the cells with the antigen in vitro and transferring the mixture to animals irradiated with X-rays. They obtained positive results only when the animals had been immunised in vivo. Our results do not confirm their findings. In our view, the main cause of their negative results is the use of a protein antigen, as the demonstration of formed antibodies by precipitation is not a very sensitive method.

The main question is whether the formation of antibodies in a transfer of a mixture of cells and antigen to young rabbits is not due to an active reaction on the part of

6

the recipients. It was demonstrated that disorganisation of the vital processes of the transferred cells—e. g. by irradiation—destroys the possibility of antibody formation after their transfer to the young animals. This does not, of course, exclude the possibility that irradiation destroys cell structures—e. g. mitochondria—which, when transferred to young animals, induce a metabolic change which makes the active reaction of the animals to the transferred antigen possible. This possibility is not supported, however, by a further finding. Following transfer of the cells, antibodies are formed in two chronologically separate phases. The first of these depends on the function of the spleen cells, while the second is the actual active response of the young rabbit to the antigen injected together with the cells. In the experimental part it was shown that the active response is not speeded up in any way as compared with injection of the antigen alone. If induction of a metabolic state occurred, making a reaction to the antigen possible, then the persistent antibody level characteristic of active formation would be determined as the first response, and not the typical curve of passive transfer. We regard the participation of the young organism in the whole reaction as passive and simply as a suitable medium for maintaining viability of the transferred cells and for the biochemical processes essential in antibody formation.

Although in experiments with Rychlíková (1957) we were not successful in demonstrating antibody formation in tissue culture, after the addition of antigen to explanted tissue, it is concluded from these experiments that the chief difference as compared with transfers to young rabbits lies in inadequate nutritional conditions in the tissue cultures. We believe that improvement of the culture medium in tissue cultures and dynamic conditions of cultivation will produce the same result—i. e. antibody formation— as in a transfer to young rabbits.

It also remains to be explained why antibody formation by transferred cells is of relatively short duration. Why does antibody formation decrease at a time when, as has been demonstrated, the transferred cells still survive in the organism of the young animal? It is possible that further generations of cells, which do not come into contact with the antigen, do not form antibodies and that the metabolic change (the production of antibodies) is not inherited by further generations of cells. In this association it should also be borne in mind that this may be the manifestation of a transplantation immunity response on the part of the recipient. Further experiments are being carried out in an attempt to determine the basis of this phenomenon.

The finding that antibodies can be produced by isolated cells under suitable conditions provides an answer to a number of questions. It defines the importance of neurohumoral factors, primarily in the formation of the most suitable metabolic conditions of the environment and of the metabolic level of the cells. On the other hand, these experiments exclude the direct causal participation of nervous factors and others in antibody formation. They are also an experimental reply to present discussion on the significance of reflex processes for the formation of antibodies. The finding of antibody formation in isolated cells permits attention to be concentrated on the stage of their development in which antibodies cannot be demonstrated serologically either in cells or in serum. We are now embarking on these investigations by studying metabolic changes which take place following contact between antigen and cells and by studying the influence of antimetabolites and radiation on the antibody reaction at cell level. At the same time the question arises as to whether it will prove possible to bring about antibody formation following the mixing of cell particles with the antigen in vitro. We raise this question because antibody formation was transferred from immunised animals to young rabbits by isolated mitochondria (Šterzl and Hrubešová 1955a).

7

Summary

Cells isolated from the spleen of an adult rabbit and mixed with an antigen in vitro *(S. paratyphi B.)* form antibodies when injected intraperitoneally in 5-day-old rabbits. At that age young rabbits do not respond to the transferred antigen by antibody formation.

No differences were found in the degree of formation, whether the young rabbits were injected with the cells of a normal rabbit, the spleen cells of a rabbit immunised with a non-specific antigen (guinea-pig serum) or with the specific antigen *(S. paratyphi B.)*, after mixing with the antigen.

Antibody formation takes place in the presence of the optimal quantitative relationship when mixing the cells and antigen in vitro. In a corpuscular antigen, two micro-organisms of *S. paratyphi B* are added to one spleen cell. Larger doses of the antigen inhibit antibody formation.

If isolated cells are irradiated, a dose of 860 r does not inhibit antibody formation, while doses of 1,000—1,200 completely inhibit it. The transferred spleen cells of an adult rabbit do not induce an active response to the antigen in young rabbits. The participation of the recipient (the young rabbit) in antibody formation is regarded as passive, i. e. the animal is regarded as a suitable culture medium for the transferred cells.

Cells injected into the organism survive; antibody formation can be evoked by the injection of the antigen in young rabbits 24 and 72 hours after the injection of washed spleen cells alone. Antibodies are also formed if the cells are injected intraperitoneally and the antigen is injected into the blood stream.

On mixing the cells with the antigen at 37 °C, the effective amount of antigen is rapidly bound by the cells. The washing out of superfluous antigen following incubation does not prevent the formation of antibodies.

References

A s k o n a s, B. A., H u m p h r e y, J. H.: Antibody Formation in Slices of Granulomata .Produced by Adjuvant. Biochem. J. 60 : X, 1955.

F a g r a e u s, A.: The Plasma Cellular Reaction and its Relation to the Formation of Antibodies in vitro. J. Immunol. 58 : 1, 1948a.

F a g r a e u s, A.: Antibody Production in Relation to the Development of Plasma Cells. Acta med. scand. Suppl. 204, 1948b.

F a s t i e r, L. B.: An Attempt to Produce Bacterial Agglutinins in vitro. J. Immunol. 60 : 399, 1948.

H a r r i s, S., H a r r i s, F. N.: Studies on the Transfer of Lymph Node Cells. V. Transfer of Cells Incubated in vitro with Suspensions of Shig. paradysenteriae. J. Immunol. 74 : 318, 1955.

H o l u b, M.: Kvantitativní změny lymfatické tkáně během imunisace. Čs. morfologie 5 1957.

M a k i n o d a n, T., R u s h, R. F., W o l f e, H. R.: Precipitin Production in Chickens. X. Cellular Changes in the Spleen During Antibody Production. J. Immunol. 72 : 39, 1954.

M a r s h a l l, A. H. A., W h i t e, R. G.: Reaction of the Reticular Tissue to Antigens. Brit. J. Exp. Pathol. 31 : 157, 1950.

M e y e r, K., L o e w e n t h a l, H.: Untersuchungen über Anaphylaxie an Gewebekulturen. Zschr. Immunitätsforsch. 54 : 420, 1927.

P a r k e r, R. C.: Studies on the Production of Antibodies in vitro. Science 85 : 292, 1937.

R o b e r t s, J. C., D i x o n, J. F.: The Transfer of Lymph Node Cells in the Study of the Immune Response to Foreign Proteins. J. Exp. Med. 102 : 379, 1955.

R y c h l í k o v á, M., Š t e r z l, J.: Pokusy o tvorbu protilátek v tkáňové kultuře. Čs. biologie, 6, 1957 (in press).

R a n e y, H. M., L o n d o n, M.: Antibody Formation in Surviving Tissues. Fed. Proc. 10 : 562, 1951.

S a l l e, A. J., M c O m i e, W. A.: Immunological Responses of Tissues Cultivated in vitro. J. Immunol. 32 : 157, 1937.

8

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S e l m a r, E.: On the Formation of Bacterial Antibodies in Tissue Cultures. Acta pathol. microbiol. scand. 21 : 517, 1944.

S t a v i t s k y, A. B.: In vitro Production of Diphtheria Antitoxin by Tissues of Immunized Animals. I. Procedure and Evidence for General Nature of Phenomenon. J. Immunol. 75:214, 1955.

Š t e r z l, J.: Průkaz a biologické vlastnosti prekursoru serových protilátek. Čs. biologie 4 : 321, 1955.

Š t e r z l, J.: The Demonstration and Biological Properties of the Tissue Precursor of Serum Antibodies. Fol. biol. (Praha) 1 : 193, 1955.

Š t e r z l, J., H r u b e š o v á, M.: Přenos tvorby protilátek nukleoproteidovými frakcemi na neimunisované příjemce. Čs. biologie 4 : 600, 1955.

Š t e r z l, J., H r u b e š o v á, M.: The Transfer of Antibody Formation by Means of Nucleoprotein Fractions to Non-immunized Recipients. Fol. biol. (Praha) 2 : 21, 1956a.

Š t e r z l, J., H r u b e š o v á, M.: Tvorba protilátek — model adaptivní proteosynthesy. Sjezd o bílkovinách 1. 12. 1955b. Čs. gastroenterol. 10 : 228, 1956b.

T a n a k a, A. H.: Studies of Antibody-producing Cells. II. The Agglutinin Formation in the Bone-marrow of Rabbits. Jap. Journ. Bact. 8 : 193, 1953.

T h o r b e c k e, G. J., K e u n i n g, F. J.: Antibody Formation in vitro by Haemopoietic Organs after Subcutaneous and Intravenous Immunization. J. Immunol. 70 : 129, 1953

Образование антител изолированными клетками селезенки после смешения с антигеном in vitro

Я. ШТЕРЦЛЬ

Резюме

Клетки, изолированные из селезенки взрослого кролика и смешанные с антигеном in vitro (S. paratyphi B) образуют антитела при впрыскивании в полость брюшины 5-дневным кроликам. В этом возрасте крольчата не реагируют на введение антигена образованием антител.

Не наблюдалось различий в образовании антител в случаях, когда крольчатам вводились после их смешения с антигеном: клетки нормального кролика, клетки из селезенки кролика, иммунизированного неспецифическим антигеном (сывороткой морской свинки) или же специфическим антигеном (S. paratyphi B).

Образование антител обусловлено оптимальным количественным соотношением смеси клеток и антигена in vitro. У корпускулярного антигена мы на 1 клетку селезенки прибавляем 2 микробов S. paratyphi B. Более значительные дозы антигена подавляют образование антител.

Если изолированные клетки облучаются 860 r, это не подавляет образования антител. Облучение клеток 1000—1200 r нарушает образование антител. Перенесение клеток селезенки взрослого кролика не вызывает активной реакции на антиген у крольчат. Мы рассматриваем участие реципиента (молодого животного) в образовании антител как пассивное, — как участие благоприятной культивационной среды для переносимых клеток.

Введенные в организм клетки выживают: образование антител можно вызвать путем впрыскивания молодым животным антигена через 24 и 72 часа после введения только промытых клеток селезенки. Антитела образуется и тогда, если клетки вводят в полость брюшины, а антиген — в кровяное русло.

При смешивании клеток с антигеном при 37° С связывание эффективного количества антигена клетками происходит быстро. Вымывание остатков антигена после инкубации не нарушает образования антител.

9